SUPPLEMENTARY MATERIALS AND METHODS

Recombinant CHIKV Plasmids

Codon-optimized C-terminal FLAG-tagged cDNA clones encoding for CHIKV capsid, E2 and E1 were generated (Genscript Corporation) and sub-cloned into pcDNA3.1 expression vector (Invitrogen) to form the pcDNA-C-FLAG, pcDNA-E2-FLAG, and pcDNA-E1-FLAG expression plasmids respectively. Positive clones containing full-length inserts were screened by restriction analysis and confirmed by DNA sequencing.

Transient transfection

Recombinant CHIKV structural proteins were expressed in HEK 293T cells as described previously (Song & Lahiri, 1995) with modifications. Cells were transfected (20 μg of plasmid DNA per 5 x 10^6 cells) using $CaPO_4$ as described (Song & Lahiri, 1995). At 24 hours post-transfection, cells were washed with PBS and lysed with ice-cold lysis buffer (20 mM Hepes, pH 7.5, 280 mM KCl, 1 mM EDTA, 10% glycerol, 1% NP-40) containing protease inhibitors (20 mM NaF, 0.1 mM Na_3VO_3, 1 mM DTT, 1 mM PMSF). Cell lysates were mixed with Laemmli buffer and stored at -20ºC for Western blot analyses.

Virus production and purification for virion-based ELISA

Virus was propagated in VeroE6 cells and viral particles were purified by ultra-centrifugation as follows: infected culture medium was filtered with 0.45 μm filters after cell debris was removed by centrifugation at 2,000 rpm for 5 minutes at 4ºC. Clear supernatant was centrifuged at 28,000 rpm for 3 hours at 4ºC, in the presence of a 20% sucrose cushion. Supernatant was removed and virus particles were

reconstituted with 100 μl of Tris/EDTA (TE) buffer and stored in aliquots at -80°C. Purified CHIK virions were quantified by quantitative reverse transcriptase-PCR (qRT-PCR).

Western blot

Fifty μg of whole-cell lysates were loaded onto 10% SDS-PAGE gels and transferred onto nitrocellulose membranes at 180 mA for 45 minutes. Immunoblot analyses were performed with human plasma samples diluted at 1:1,000 to 1:2,000 in PBST-milk supplemented with 3% Fetal Bovine Serum (FBS) and incubated for 2 hours at room temperature. This was then followed by incubation for 1 hour at room temperature with goat anti-human IgG conjugated to HRP (Molecular Probes) diluted 1:10,000 in PBST-milk supplemented with 3% FBS, or with mouse anti-human IgG3 isotype conjugated to HRP (Molecular Probes) diluted 1:1,250 in PBST-milk supplemented with 3% FBS. Bands were visualized on X-ray films (Kodak) by chemiluminescence (Amersham Biosciences). Densitometry analysis was performed with NIH ImageJ (http://rsb.info.nih.gov/ij/) to quantify the bands obtained. Signal was recorded within the selection area and expressed as mean-grey values.

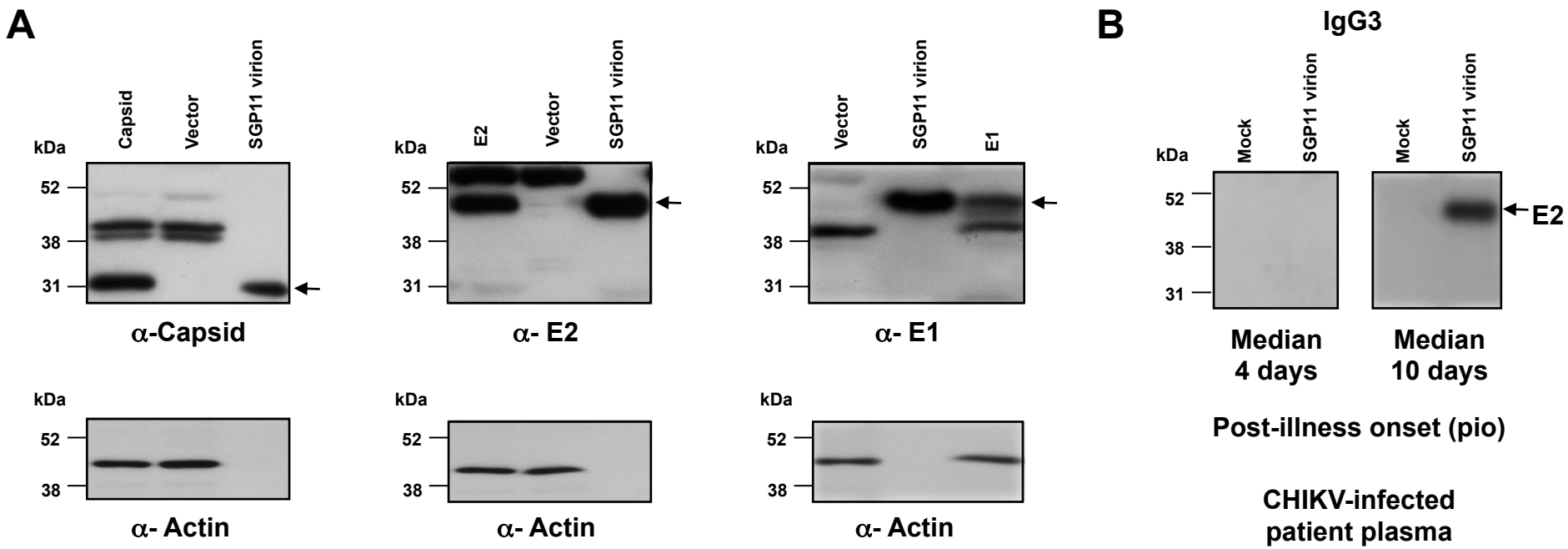
Peptide-based ELISA

Briefly, streptavidin-coated microplates (Pierce) were first blocked with 1% sodium caseinate (Sigma-Aldrich) diluted in 0.1% PBST (0.1% Tween-20 in PBS), before coating with peptides diluted at 1:1,000 in 0.1% PBST and incubated at room temperature for 1 hour on a rotating platform. Plates were then rinsed with 0.1% PBST before incubation with human plasma samples diluted at 1:200 to 1:2,000 in 0.1% PBST for 1 hour at room temperature. This was followed by incubation with the

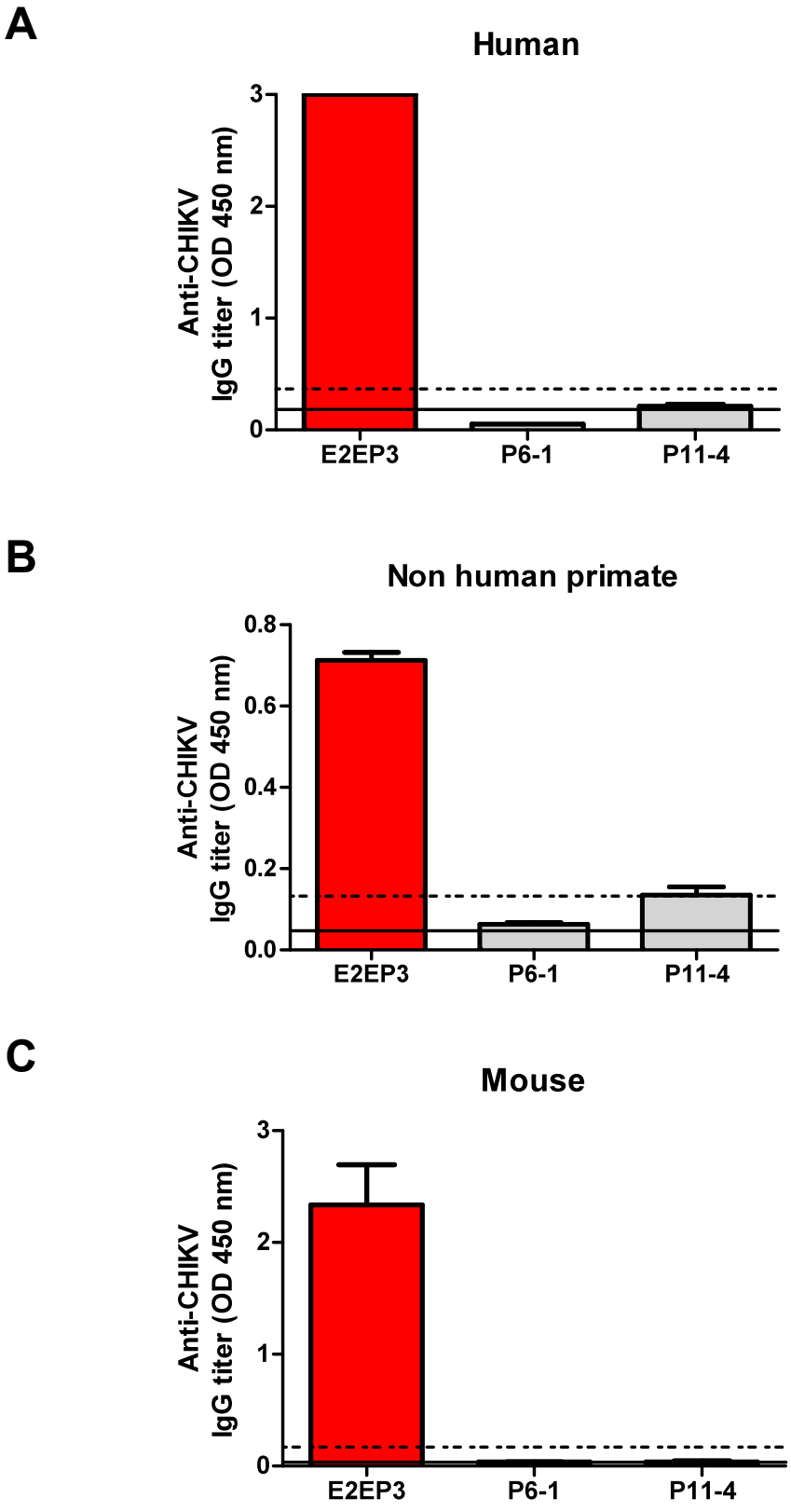
respective anti-human IgG and isotype-specific antibodies conjugated to HRP (Molecular Probes) at dilutions from 1:500 to 1:4,000 in 0.1% PBST supplemented with 0.1% sodium caseinate for 1 hour at room temperature to detect for any antibodies bound to the peptide samples. Binding was detected with TMB substrate solution (Sigma-Aldrich) and color development was stopped with STOP reagent (Sigma-Aldrich). Absorbance was measured at 450 nm using a microplate autoreader (Tecan). Peptides are considered positive if absorbance values are higher than the mean + 6 standard deviation (SD) values of negative controls.

Plaque assays

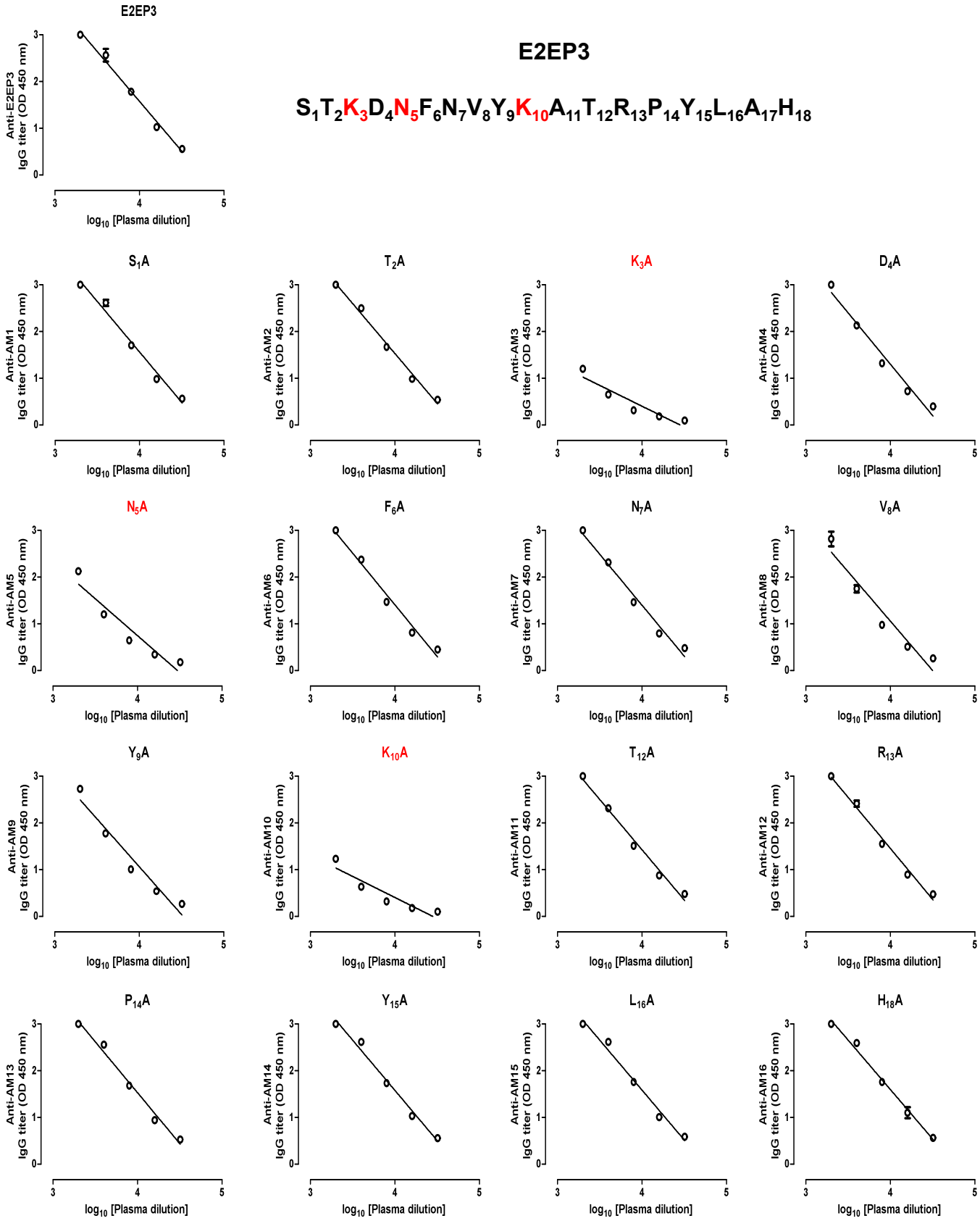
Ten μl of blood was collected from the tail of each mouse in 1 μl of citrate and 89 μl of Hank's buffer (Sigma-Aldrich) and serially diluted up to 10^{-3} times with Hank's buffer. Vero E6 cells were pre-seeded at 2.5 x 10^5 cells per well in 24-wells plate and incubated at 37ºC for 20 hours. Ninety μl of diluted virus mix was inoculated into each well and incubated for 1 hour at 37°C. Virus overlay was removed and the infected monolayers were washed once with 1ml of sterile PBS. One ml of 1 % w/v carboxymethylcellulose (Calbiochem) in DMEM with 5% FBS was then added onto the infected monolayers. Plates were incubated at 37°C with 5% CO_2 for 72 hours and visualized by staining the monolayer with 1 ml of 0.1% w/v crystal violet (Sigma-Aldrich)/ 10% v/v formaldehyde (Sigma-Aldrich) for 2 hours at room temperature.



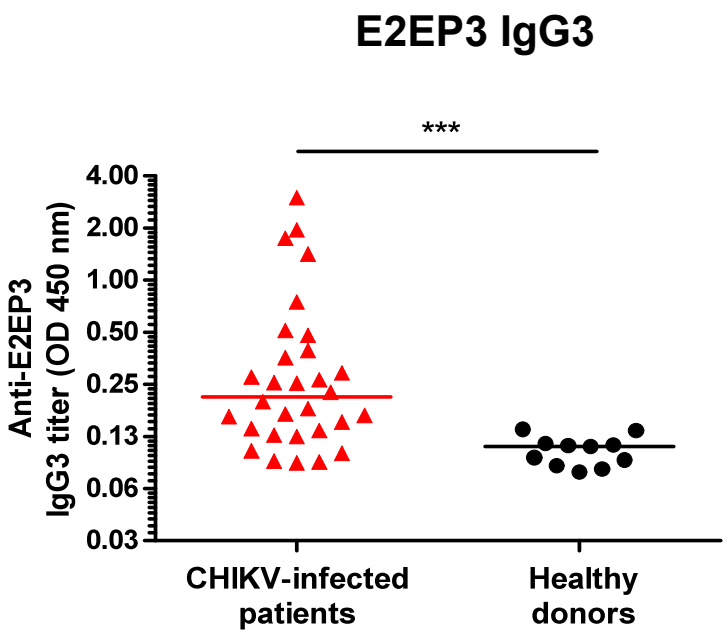
Supplementary Figure 1. Expression of CHIKV structural proteins. A. Total cell lysates were prepared from cells transiently transfected with plasmids expressing capsid (Capsid plasmid), E2 (E2 plasmid) and E1 (E1 plasmid). Vector transfected (Vector plasmid) were used as negative controls. Lysates and purified CHIKV virion (SGP11) were subjected to SDS-PAGE and probed with antigen specific polyclonal rabbit antisera (Biogenes) at a dilution of 1:2,000, followed by secondary anti-rabbit IgG HRP antibodies. B. Purified CHIKV virions were subjected to SDS-PAGE and probed with CHIKV-infected patients' plasma at 1:1,000, followed by secondary anti-human IgG3 isotype specific antibodies.



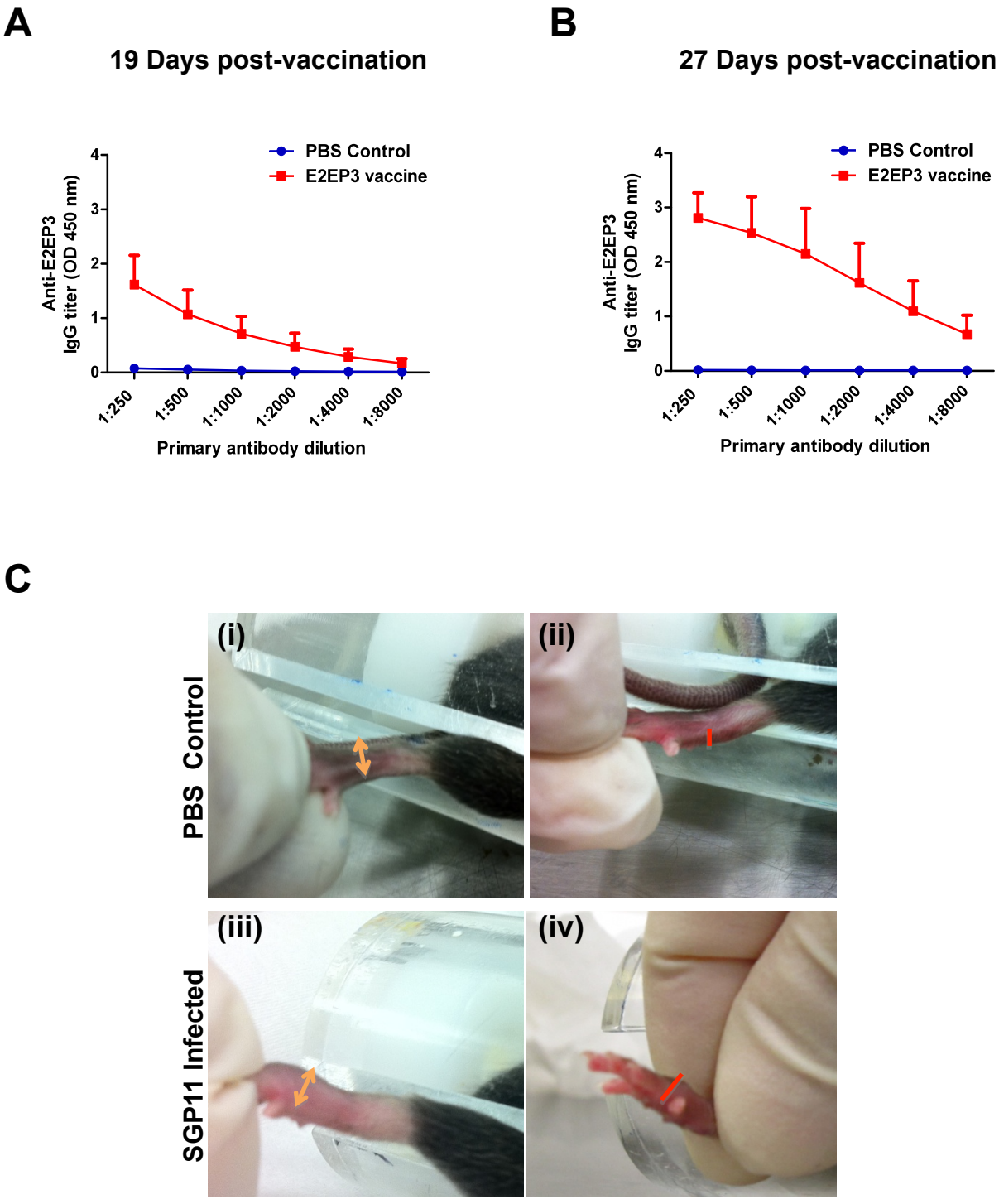
Supplementary Figure 2. Specificity of E2EP3. Individual peptides were screened using A. CHIKV-infected patient plasma pools (Median 10 days pio); B. CHIKV-infected non-human primates (NHP) plasma (13 days post-infection); and C. CHIKV-infected mouse sera (21 days post-infection). Black solid line represents the mean value of the healthy donors and controls. Dotted line represents the value of mean ± 6 SD. Results represent an average of 3 independent experiments. P6-1 represents the peptide sequence residing in E2 domain B, while P11-4 represents the peptide sequence residing in C-terminus of E2 glycoprotein (structure not resolved).



Supplementary Figure 3. Alanine-scan analysis of E2EP3 by anti-CHIKV antibodies. Alanine substitutions were constructed at each position of E2EP3 except the existing alanines. CHIKV-infected patients' plasma pools were used to validate binding capacity. Plasma pools at median 10 days pio were tested in a set of serial dilutions from 1:2000 to 1:32000 and assayed in triplicates. Results are expressed as mean ± SD. Data are representative of 3 independent experiments.



Supplementary Figure 4. Validation of E2EP3 specific IgG3 antibodies in 30 CHIKV-infected patients. Individual plasma samples at median 10 days pio were subjected to E2EP3 specific peptide-based ELISA at a dilution of 1:200, followed by secondary human anti-IgG3 isotype HRP. Healthy donors' plasma (n = 11) were used as controls. Samples assayed were performed at triplicate. ***, $P < 0.001$ by Mann-Whitney U test. The y axis is plotted in log 2 scale. Red straight line represents the median of the CHIKV-infected patients' group and black straight line represents the median of the healthy donors' group.



Supplementary Figure 5. E2EP3-induced antibody response in vaccinated mice. E2EP3 specific peptide-based ELISA was used to measure the titer after E2EP3 peptide vaccination at A. 19 days post-vaccination, and at B. 27 days post-vaccination. Mouse anti-E2EP3 IgG antibodies were detected after the second and third vaccination. All results are expressed as mean ± SD. Samples assayed were performed at triplicate. C. CHIKV-induced footpad inflammation. Effect of CHIKV injected into the footpad. (i) and (iii) represent respective photos of control and infected groups, and measurement of the width are indicated by orange arrows. (ii) and (iv) represent respective photos of control and infected groups, and measurement of the thickness are indicated by red line.